

0.4% Trypan Blue Solution

Introduction

Trypan blue staining is one of the most common methods for measuring cell viability. Trypan Blue is an azo dye and is cell membranes impermeable, so live cells cannot be stained, and damaged or dead cells can be stained to blue. Trypan Blue Staining identifies live and dead cells directly. Trypan Blue Staining is also used for cell counting and calculating cell viability.

Components and Storage

Components	K1183-50 mL	K1183-100 mL
0.4% Trypan Blue Solution	50 mL	100 mL
Store the solution at room temperature away from light. Stable for 2 years.		

Protocol

1. Cells collection

- Adherent cells:** digest cells with trypsin, 800 rpm centrifugation for 2 min, and discard the supernatant. Resuspend the cell pellets with PBS or serum-free medium.
- Suspended cells:** 800 rpm centrifugation for 2 min and discard the supernatant. Resuspend the cell pellets with PBS or serum-free medium.

***Note:** If the density of cells is too high, dilute the cell suspension before starting staining.

2. Trypan Blue staining

- Add 0.5 mL 0.4% Trypan Blue Solution to 0.5 mL cell suspension. gently mix and incubate for 30 s at room temperature.

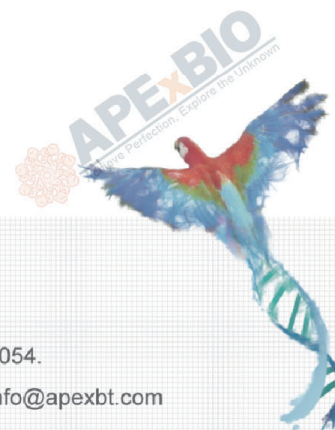
***Note:** The staining time can be adjusted. However, trypan blue has toxicity to living cells, so the staining time should not be too long.

- Add a drop of the stained cell suspension to a hemacytometer. Place the hemacytometer under the microscope for cell counting.
- Calculate the percentage of viable cells as follows:

$$\text{Viable cells (\%)} = \frac{\text{total number of viable cells}}{\text{total number of cells}} \times 100\%$$

Note

1. Trypan Blue has toxicity to living cells, so the staining time should not be too long. Viable cells are eventually stained after long-time staining.
2. When cell counting, to make sure the correction of the result, at least 500 cells are a must.
3. This solution may appear a small amount of precipitate after long-time storage. Heat this solution in a 37°C water bath to dissolve the precipitate. When the precipitate is dissolved, it can be used normally.
4. For your safety and health, please wear lab coats and gloves during the experiment.
5. For research use only. Not to be used in clinical diagnostic or clinical trials.



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